Received: 9 January 2020 | Revised: 24 April 2020 | Accepted: 28 April 2020

DOI: 10.1111/bcp.14357

ORIGINAL ARTICLE

A pharmacokinetic-pharmacodynamic study of a single dose of febuxostat in healthy subjects

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Aims: To examine the pharmacokinetic-phamacodynamic (PK-PD) relationships of plasma febuxostat and serum urate and the effect of a single dose of the drug on renal excretion and fractional clearance of urate (FCU).

Methods: Blood and urine samples were collected at baseline and up to 145 hours following administration of febuxostat (80 mg) to healthy subjects (n = 9). Plasma febuxostat and serum and urinary urate and creatinine concentrations were determined. Febuxostat pharmacokinetics were estimated using a two-compartment model with first-order absorption. An Emax PK-PD model was fitted to mean febuxostat and urate concentrations. Urinary urate excretion and FCU were calculated pre- and post-dose.

Results: Maximum mean plasma concentration of febuxostat (2.7 mg L^{-1}) was observed 1.2 hours after dosage. Febuxostat initial and terminal half-lives were 2.0 ± 1.0 and 14.0 ± 4.7 hours (mean ± SD), respectively. The majority (81%) of the drug was eliminated in the 9 hours after dosing. Serum urate declined slowly achieving mean nadir (0.20 mmol L^{-1}) at 24 hours. The IC_{50} (plasma febuxostat concentration that inhibits urate production by 50%) was 0.11 ± 0.09 mg L^{-1} (mean ± SD). Urinary urate excretion changed in parallel with serum urate. There was no systematic or significant change in FCU from baseline.

Conclusion: The PK-PD model could potentially be used to individualise febuxostat treatment and improve clinical outcomes. A single dose of febuxostat does not affect the efficiency of the kidney to excrete urate. Further investigations are required to confirm the present results following multiple dosing with febuxostat.

KEYWORDS

clinical pharmacology, febuxostat, gout, pharmacokinetic-pharmacodynamic, rheumatology

1 | INTRODUCTION

Febuxostat is a selective inhibitor of xanthine oxidoreductase (XOR) thereby reducing concentrations of uric acid (urate) in serum and is indicated for the treatment of gout. The majority of the pharmacokinetic studies have estimated the pharmacokinetic parameters of febuxostat using standard noncompartmental methods following single and multiple doses administered to healthy subjects.[1–11] The

PI statement: The authors confirm that the Principal Investigator for this paper is Professor Richard Day and that he had direct clinical responsibility for the study subjects.

pharmacokinetics of the drug have also been described using a one-compartment population pharmacokinetic model.[12] However, visual inspection of the time course of plasma febuxostat concentrations suggests a two-compartment pharmacokinetic model would fit the data better.[13] A two-compartment population pharmacokinetic model was used to estimate the pharmacokinetic parameters of febuxostat in a study published in abstract form only.[14] In this study a high coefficient of variation (71%) with respect to the prediction of the plasma febuxostat concentrations was reported.[14] Thus, the pharmacokinetics of febuxostat have not been sufficiently elucidated and vary substantially in the limited published reports.

To date, there has been very limited study of the time course of the hypouricaemic effects following single doses of febuxostat. Serum concentrations of urate decline slowly and recover slowly after dosage with febuxostat. For example, Zhang et al. (2014) showed that serum concentrations of urate were reduced by 25%, 38% and 55% at around 24 hours following single doses of febuxostat of 40, 80 and 120 mg, respectively, and returned to baseline concentrations 48 hours after dosing.[11] Similarly, single doses of febuxostat of 40, 80, 120 and 240 mg have been shown to achieve maximum reductions in serum urate concentrations from baseline of 18%, 24%, 28% and 36% in normouricaemic subjects.[15] In contrast to the study of Zhang et al. (2014),[11] serum urate concentrations had not yet returned to baseline 60 hours after the dose.[15] More detailed studies are required to better understand the time course of the relationship between plasma febuxostat and serum urate concentrations.

Liu et al. (2017) reported that multiple dosing with febuxostat reduced the fractional clearance of urate (FCU) in both healthy and gouty subjects.[16] This suggests that the hypouricaemic effects of febuxostat due to inhibition of XOR may be reduced to some degree by decreased clearance of urate. This may be important in understanding the dose-response of febuxostat and consequently in establishing therapeutic doses of this drug. By comparison, allopurinol, also a XOR inhibitor, does not affect the FCU after either single[17] or multiple doses.[18] Prospective studies are required to investigate this possible effect of febuxostat on the FCU.

The aims of this study were therefore to examine (i) the pharmacokinetic-pharmacodynamic (PK-PD) relationship between plasma febuxostat and serum urate concentrations following a single dose of febuxostat and (ii) the effect of a single dose of febuxostat on both the renal excretion of urate and the FCU.

What is already known about this subject

- The pharmacokinetics of febuxostat have been described mainly using standard noncompartmental analysis.
- There are limited data describing the pharmacokinetic-pharmacodynamic (PK-PD) relationships of febuxostat.
- Based on a previous multiple dose study, febuxostat has been reported to reduce the fractional renal clearance of urate (FCU) in healthy and gouty subjects.

What this study adds

- The pharmacokinetics of febuxostat have been elucidated using a two-compartment model. The PK-PD relationship has been described with an E-max equation.
- Febuxostat displayed a negative hysteresis, with the change in serum urate concentrations lagging behind the time course of plasma febuxostat.
- A single dose of febuxostat reduced the rate of excretion of urate but did not reduce the FCU in healthy subjects.

2 | METHODS

2.1 | Study design

This was an open-label, observational study conducted in healthy subjects. The study (SVH 15/276) was approved by the Human Research Ethics Committee of St Vincent's Hospital, Sydney and registered (ACTRN 12617001346369). All study participants provided oral and written consent.

The study was conducted in two stages 12 weeks apart. Stage I was undertaken in two healthy male subjects to ascertain whether the sample collection time frame and frequency of individual collections satisfactorily captured the time course of concentrations of urate until their return to baseline values. Blood (5 mL) was collected immediately pre-dose (baseline) and at the following time points after administration: 1, 1.5, 2, 4, 6, 9, 24 (±2), 30 (±2), 31 (±2), 48 (±2) and up to 145 hours. Having demonstrated a sufficient duration of blood sampling (i.e. serum urate concentrations returned to baseline levels 145 hours after drug administration) and to also examine the effect of a single dose of febuxostat (80 mg) on the renal excretion and clearance of urate, the above two male subjects underwent the following modified study procedures. Blood (5 mL) was collected immediately pre-dose (baseline) and at the following time points after administration: 1, 3, 6, 9, 24 (±2), 31 (±1), 48 (±2), 72 (±2), 96 (±2) and 102 hours. Urine was collected before the administration of febuxostat (−24 to −12 hours and −12 to 0 hours) and at the following times after drug administration: 0-2, 2-4, 4-6, 6-8, 8-12, 12-24, 25 (±1), 31 (±1), 48 (±2), 72 (±2), 96 (±2) and 101.5-102.5 hours. The 31, 48, 72 and 96 hour samples were spot urine collections while the remainder were total urine collections. The aim was to collect the blood samples at the mid-points of urine collections to obtain urate clearance estimates. In Stage II of the study, a further seven healthy participants (four males and three females) were administered a single dose of febuxostat 80 mg under fasting conditions. Blood (5 mL) was collected immediately pre-dose (baseline) and at the following time points after administration: 1, 3, 6, 9, 24 (±2), 31 (±1), 48 (±2), 72 (±2), 96 (±2) and 102 hours. Timed and divided whole urine collections continued for the duration of the study up to 102 hours after the administration of febuxostat 80 mg.

Subjects were asked to abstain from alcoholic beverages and caffeinated drinks for at least 24 hours prior to the pre- dose urine collections (i.e. 48 hours prior to taking febuxostat) and until study completion. Participants were asked to control their fluid/water intake to a rate of 100 mL per hour during the daytime.

2.2 | Bioanalysis

All blood samples were immediately centrifuged (4,000 rpm, 5 min, 20°C) and the plasma and serum were stored at −20°C until analysis. An aliquot (5 mL) of the urine samples was stored at 8°C until analysis. Plasma concentrations of febuxostat were determined using a validated HPLC assay method with a lower level of quantitation of 0.005 mg L^{-1}.[19] In this method, the standard curves were linear over the concentration range 0.005 to 10.00 mg L^{-1}. The accuracy and imprecision for the back calculated standard concentrations were 95-107% and <9.5%, respectively, with a coefficient of determination $r^2 > 0.99$. The inter-day accuracy and imprecision of the quality control samples (0.0075, 0.015, 3.00 and 9.80 mg L^{-1}) and the lower limit of quantification were within 90-115% and ≤14.5%, respectively. Method validation and criteria were in accordance with the FDA guidance on bioanalytical method validation.[20] Febuxostat concentrations in urine were not measured. Urate and creatinine concentrations in serum and urine were determined by the uricase[21] and Jaffe[22–24] methods, respectively, in the Sydpath Laboratory, St Vincent's Hospital, these assays were subject to routine validation for precision and accuracy.

2.3 | Pharmacokinetic analysis

Pharmacokinetic parameters for each subject. A two-compartment model was used to fit the time courses of plasma febuxostat concentrations for each individual using logarithmic least squares using MINIM version 3.0.8[25] as given by Equation 1:

$$\mathrm{Cp} = A \times \exp(-\alpha t) + B \times \exp(-\beta t) - (A + B) \times \exp(-k_a t) \quad (1)$$

where *Cp* is the plasma concentration of febuxostat, *A* and *B* are concentration terms, α and β are rate constants of elimination in the initial and terminal phases, and k_a is the rate constant of absorption.

Due to the sparse sampling in the absorption phase, the value of k_a was fixed at 2 h^{-1}. This value of k_a is similar to that assumed (k_a = 2.18 h^{-1}) by Hirai et al. (2016).[26] The pharmacokinetic parameters were determined using standard pharmacokinetic equations.[27–29] The estimated apparent clearance of febuxostat in each subject was normalised for body weight.

2.4 | Time course of urate concentrations

The serum urate nadir, the time to reaching the nadir, absolute and percentage reduction in serum urate concentrations, and time to recovery from the nadir were determined in each individual.

2.5 | Pharmacokinetic-pharmacodynamic analysis

The plasma febuxostat concentrations from the nine individual subjects at times 0, 1, 3, 6, 9, 24, 30, 48, 72, 96 and 102 hours were averaged. Then a two-compartment model (Equation 1) was fitted to provide the averaged time course for the cohort with overall values for *A*, *B*, α and β. The serum urate concentrations from all the nine individuals at the same time points were also averaged. Equation 2 was fitted to the average concentrations of febuxostat and urate at each time point using MINIM version 3.0.8.[25]

$$\mathrm{Cu}_t = \mathrm{Cu} \times \exp(-k_u \times t \times (\mathrm{Cp}_t / (\mathrm{IC}_{50} + \mathrm{Cp}_t))) \quad (2)$$

Cu_t is the serum urate after dosage with febuxostat at time (*t*), *Cu* is the pre-dose concentration of serum urate. Cp_t is the plasma concentration of febuxostat at time (*t*), IC_{50} is the plasma concentration of febuxostat when the production of urate is inhibited by 50% and k_u is the rate constant of elimination of urate. Equation 2 predicts a biphasic time course of urate concentrations following a single dose, initially a decline and then recovery of urate as a function of the time variant factor $Cp_t/(IC_{50} + Cp_t)$.

2.6 | Renal excretion and clearance of urate

A number of estimates of the renal excretion and clearance of urate were undertaken, repeating the estimates made in the study of Liu et al. (2017) on febuxostat. These estimates were calculated for each timed urine collection and for spot urine collections.

The urinary urate excretion rate was estimated as:

$$E_{\mathrm{UR}} = \frac{U_{\mathrm{UR}} \times V}{\mathrm{time}} \quad (3)$$

The ratio of the urinary urate to creatinine excretion rates was estimated as:

$$\frac{E_{\mathrm{ur}}}{E_{\mathrm{cr}}} = \frac{U_{\mathrm{ur}} \times V}{\mathrm{time}} \div \frac{U_{\mathrm{cr}} \times V}{\mathrm{time}} = \frac{U_{\mathrm{ur}}}{U_{\mathrm{cr}}} \quad (4)$$

The Simkin Index (SI),[30] a measure of the efficiency of the kidney in clearing urate, is:

$$\mathrm{SI} = \frac{U_{\mathrm{ur}} \times S_{\mathrm{cr}}}{U_{\mathrm{cr}}} \quad (5)$$

and the FCU[31] is:

$$\mathrm{FCU} = \left(\frac{U_{\mathrm{ur}} \times V}{S_{\mathrm{ur}} \times \mathrm{time}} \Big/ \frac{U_{\mathrm{cr}} \times V}{S_{\mathrm{cr}} \times \mathrm{time}}\right) \times 100 = \left(\frac{U_{\mathrm{ur}} \times S_{\mathrm{cr}}}{S_{\mathrm{ur}} \times U_{\mathrm{cr}}}\right) \times 100 \quad (6)$$

In these equations, *E* is the excretion rate (mmol h^{-1}), U_{cr} and U_{ur} are the urinary creatinine and urate concentrations (mmol L^{-1}), *V* is volume of urine (L), time is the duration of the urine collection (h), S_{cr} and S_{ur} are serum creatinine and urate concentrations (mmol L^{-1}), and FCU is the fractional renal clearance of urate (%).

2.7 | Statistical analysis

The data are presented for the total study cohort as well as separately in the male and female subjects. The pre- and post-dose FCU values were compared using signed rank Wilcoxon tests. The crude *P* values of the Wilcoxon tests have been adjusted using the Bonferroni corrections. Pharmacokinetic and pharmacodynamics data from the two subjects who underwent the study twice were averaged. Statistical tests and curve fitting were performed using R statistical programming software version 3.4[32] and MINIM version 3.0.8,[25] respectively. Plots were generated using Graphpad version 7.0.3.[33]

3 | RESULTS

3.1 | Baseline characteristics

The study comprised nine subjects: six males and three females. The baseline characteristics were similar for male and female subjects (Table 1) with the exception of serum urate concentrations, which were lower in female than male subjects ($P < 0.05$).

3.2 | Pharmacokinetics of febuxostat

Febuxostat reached a maximum plasma concentration of 2.7 ± 0.9 mg L^{-1} (mean ± SD) at 1.2 h ± 0.6 hours following oral administration of a single dose of 80 mg to fasting healthy subjects (Figure 1a). A two-compartment pharmacokinetic model fitted the individual time courses of the plasma concentrations of febuxostat ($r^2 > 0.97$; Table 2). There was a large decline in the plasma concentrations of febuxostat by up to 100-fold over 24 hours. The mean initial and terminal half-lives from the two-compartment model were 2 and 14 hours, respectively from the analysis of individual subjects. The area under the concentration-time curve (AUC) of the initial (alpha) phase, from 0 to 9 hours post dosing ($AUC_{0\text{-}9}$), comprised 81% of the total AUC to infinity ($AUC_{0\text{-}\infty}$). The $AUC_{0\text{-}t}$ where *t* is the last measured concentration was very close to the $AUC_{0\text{-}\infty}$. Although the apparent clearance of febuxostat in females tended to be lower than in male subjects, this contrast disappeared when the apparent clearance was normalised for body weight (Table 2).

3.3 | Effect of a single dose of febuxostat on the time course of serum urate concentrations

The hypouricaemic effect of febuxostat was evident within an hour of drug administration. The nadir of serum urate was higly variable but was achieved at an average of 24 ± 16 hours after dosage (Table 3). Serum urate concentrations recovered gradually to near the baseline concentrations over about 3 days (Figure 1b). The maximal absolute and percentage reductions of serum urate concentrations were 0.10 ± 0.02 mmol L^{-1} and 34 ± 11%, respectively. The percentage reduction in serum urate concentrations was higher in females compared to males (47% versus 29%, $P < 0.05$; Table 3).

3.4 | Pharmacokinetic-pharmacodynamic relationship of a single dose of febuxostat

When the changes in the concentrations of serum urate from baseline were plotted against the plasma concentrations of febuxostat at

TABLE 1 Baseline characteristics of the healthy subjects (n = 9) administered a single dose of febuxostat (80 mg)

	Male subjects (n = 6)	Female subjects (n = 3)	Total (n = 9)
Age (year)	26 ± 5	25 ± 2	26 ± 4
Weight (kg)	73 ± 10	63 ± 12	70 ± 12
Body mass index (kg m^{-2})	24 ± 2	23 ± 5	24 ± 3
Body surface area (m^2)	1.9 ± 0.2	1.7 ± 0.1	1.8 ± 0.2
Serum urate concentration (mmol L^{-1})[a]	0.32 ± 0.03	0.23 ± 0.04	0.28 ± 0.05
eGFR (mL/min/1.73 m^2)	114 ± 6	115 ± 16	114 ± 10
Urate excretion rate (mmol h^{-1})	0.18 ± 0.06	0.13 ± 0.04	0.17 ± 0.06
Ratio of urate excretion rate to creatinine excretion rate	0.25 ± 0.05	0.28 ± 0.07	0.26 ± 0.06
Simkin index (mmol L^{-1})[b]	0.023 ± 0.004	0.019 ± 0.006	0.020 ± 0.005
FCU (%)	6.5 ± 1.5	8.1 ± 1.5	7.0 ± 1.7
Urine flow rate (L h^{-1} kg^{-1})	0.9 ± 0.3	1.3 ± 0.2	1.1 ± 0.3

Data presented as mean ± SD

[a]Males compared with females with unpaired Wilcoxon tests. Statistical significance ($P < 0.05$); $P = 0.03$.

[b]Simkin Index (SI) = the urinary excretion rate of urate over creatinine clearance ($SI = \frac{U_{ur} \times S_{cr}}{U_{cr}}$) in mmol L^{-1}.

Abbreviations: eGFR, estimated glomerular filtration rate in mL min^{-1} 1.73 m^{-2} as calculated by The Chronic Kidney disease Epidemiology Collaboration (CKD-EPI) 2009 equation;[34] FCU, fractional renal clearance of urate in %.

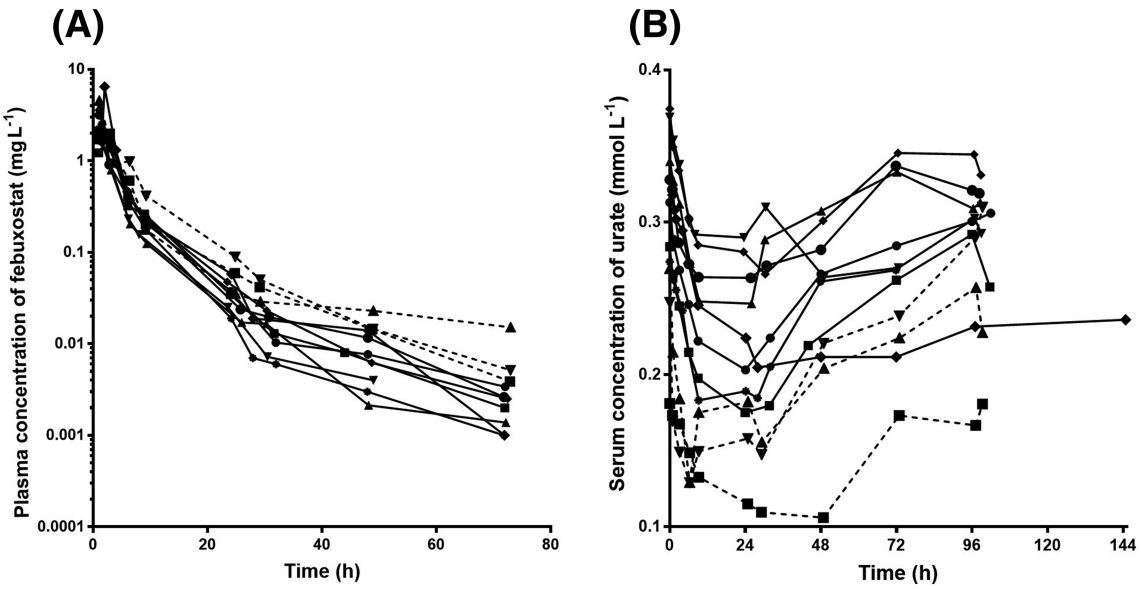

FIGURE 1 A, The time courses of the plasma concentrations of febuxostat and B, serum urate concentrations in healthy subjects (n = 9) following the administration of a single dose of febuxostat (80 mg). Dashed lines are female subjects and solid lines are male subjects. Each individual has a unique symbol

TABLE 2 Pharmacokinetic parameters of febuxostat following the administration of a single dose (80 mg) in individual healthy subjects (n = 9)

Pharmacokinetic parameter	Male subjects (n = 6)	Female subjects (n = 3)	Total (n = 9)
C_{max} (mg L^{-1})	2.8 ± 0.8	2.7 ± 1.3	2.7 ± 0.9
T_{max} (h)	1.4 ± 0.8	1.0 ± 0.06	1.2 ± 0.6
AUC (mg h L^{-1}) in α phase	8.1 ± 1.4	10.1 ± 2.0	8.8 ± 1.9
AUC (mg h L^{-1}) in β phase	2.0 ± 0.7	2.8 ± 0.8	2.3 ± 0.9
$AUC_{0\text{-}t}$ (mg h L^{-1})	10.1 ± 1.8	12.9 ± 1.6	11.0 ± 2.2
$t_{1/2}$ (h) in α phase	1.7 ± 0.5	2.6 ± 1.3	2.0 ± 1.0
$t_{1/2}$ (h) in β phase	12.2 ± 3.4	17.7 ± 4.9	14.0 ± 4.7
CL/F (L h^{-1})	8.2 ± 1.6	6.3 ± 0.7	7.6 ± 1.6
CL/F (L h^{-1} kg^{-1})	0.11 ± 0.03	0.10 ± 0.01	0.11 ± 0.02

Data presented as mean ± SD.
α and β (hybrid rate constants) are rate constants (h^{-1}) in the initial and terminal phases of febuxostat elimination, respectively,
Abbreviations: $AUC_{0\text{-}t}$ = area under the concentration-time curve to the last measured concentration; C_{max}, maximum concentration; CL/F, apparent clearance.

TABLE 3 Pharmacodynamic effect (serum urate lowering effect) of a single dose of febuxostat (80 mg) in healthy subjects (n = 9)

	Male subjects (n = 6)	Female subjects (n = 3)	Total (n = 9)
Nadir serum urate concentration (mmol L^{-1})	0.23 ± 0.02	0.12 ± 0.01	0.20 ± 0.06
Time (h) to nadir (maximum effect)	25.0 ± 4.8	20.5 ± 11.6	24.3 ± 15.6
Absolute reduction in urate (mmol L^{-1})	0.09 ± 0.01	0.11 ± 0.02	0.10 ± 0.02
Reduction in serum urate concentration (%)[a]	29.0 ± 2.8	47.0 ± 2.5	34.0 ± 11.1
Time (h) to recovery from the nadir[b]	69.6 ± 21.0	69.3 ± 16.6	66.7 ± 17.4

Data presented as mean ± SD.
[a]Males compared with females with unpaired Wilcoxon tests. Statistical significance ($P < 0.05$); $P = 0.03$.
[b]Recovery was defined as the time serum urate concentrations returned to baseline concentrations.

sequential times, a negative hysteresis was apparent for all individual subjects and also in the mean concentrations of febuxostat and urate. At the highest plasma concentrations of febuxostat there was minimal change in the concentrations of serum urate (Figure 2 and Supporting Information Figure S1). By contrast, the maximal change in serum urate was apparent approximately 24 hours after the majority of febuxostat had been eliminated from plasma (Figure 2).

3.4.1 | Phamacokinetic-pharmacodynamic model of mean plasma febuxostat and serum urate concentrations.

The PK-PD model described by Equation 2 fitted the time course of mean plasma febuxostat and serum urate concentrations from the study subjects (n = 9) well (r^2 > 0.99; Figure 3 and Table 4). The rate constant of elimation of urate, k_u, was 0.053 h^{-1}, equivalent to a half-life of 13 hours. The mean IC_{50} value was 0.11 mg L^{-1} (Figure 3). This concentration is considerably lower than the mean peak plasma concentrations of febuxostat observed (2.7 ± 0.9 mg L^{-1}).

3.5 | Effect of febuxostat on the renal excretion and clearance of urate

The ratio of the urinary urate excretion rate to creatinine excretion rate decreased in parallel with the serum urate concentrations and then increased gradually over 3 days as serum urate concentrations returned towards baseline concentrations (Figures 1b and 4a). The other estimates of renal excretion of urate, namely the rate of urate excretion, the Simkin Index that normalises the urinary excretion rate of urate to the creatinine clearance and the sequential 24 hour urinary urate excretion collections, gave similar patterns, mirroring the changes in serum urate (Supporting Information Figure S2a-c) By contrast to the estimates of urate excretion, there was no discernible pattern of change in FCU after the administration of a single dose of febuxostat (Figure 4b and Table 5). There was no statistically significant change in FCU in comparison to the baseline FCU at any time post dosing with febuxostat (Table 6). There was no effect of febuxostat on the renal clearance of urate over baseline (Supporting Information Table S1). Similarly, there was no change in renal creatinine clearance compared to baseline following the single dose of febuxostat (Supporting Information Table S2).

4 | DISCUSSION

The C_{max} and T_{max} of febuxostat were similar between male and female subjects and consistent with published data.[1,4–6,11] The two-compartment pharmacokinetic model fitted the plasma febuxostat concentrations well both for individual subjects and the averaged concentration-time data. The apparent clearance of febuxostat tended to be lower in females compared to males, but this contrast was not significant when the apparent clearance of febuxostat was normalised for body weight (Table 2). A similar observation was made by Liu et al. (2012), who reported that, although the apparent clearance of febuxostat was lower in healthy Chinese compared to North Americans, this difference was not significant when normalised for body weight.[7,10]

Febuxostat exhibited a negative hysteresis, in keeping with the slow decline in serum concentrations of urate that lagged behind the rapid fall in the plasma concentrations of febuxostat. It is notable that the nadir of serum urate was achieved at about 24 hours when the bulk of febuxostat had been eliminated from plasma. The delayed time

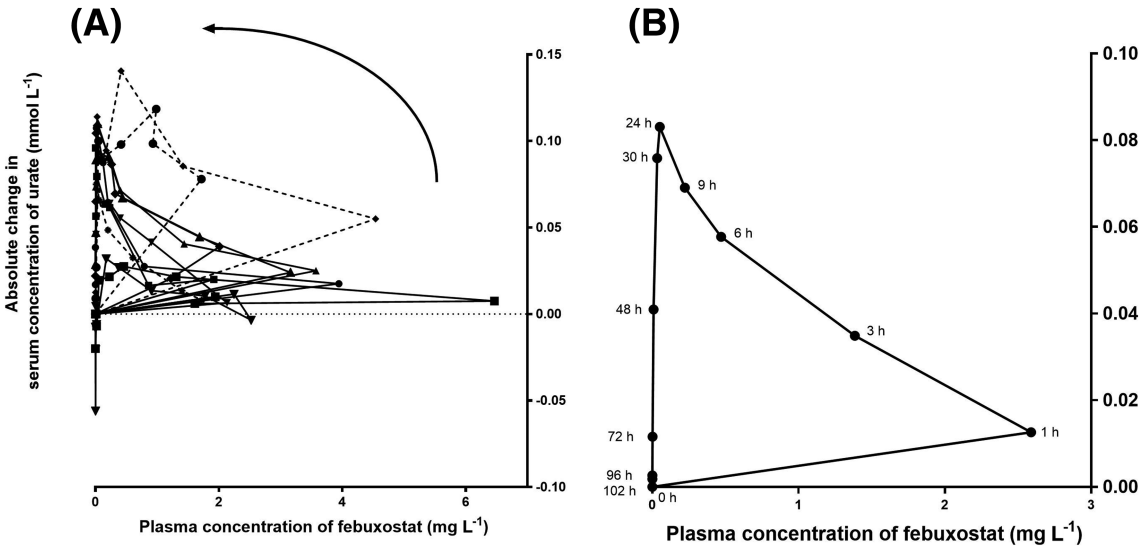

FIGURE 2 Time course of the relationship between plasma febuxostat concentration and absolute change in serum urate concentration from time zero demonstrating a negative hysteresis in A, all individual subjects and B, mean concentrations from all subjects derived from the PK-PD model. The equivalent data from a single representative subject is presented in Supporting Information Figure S1

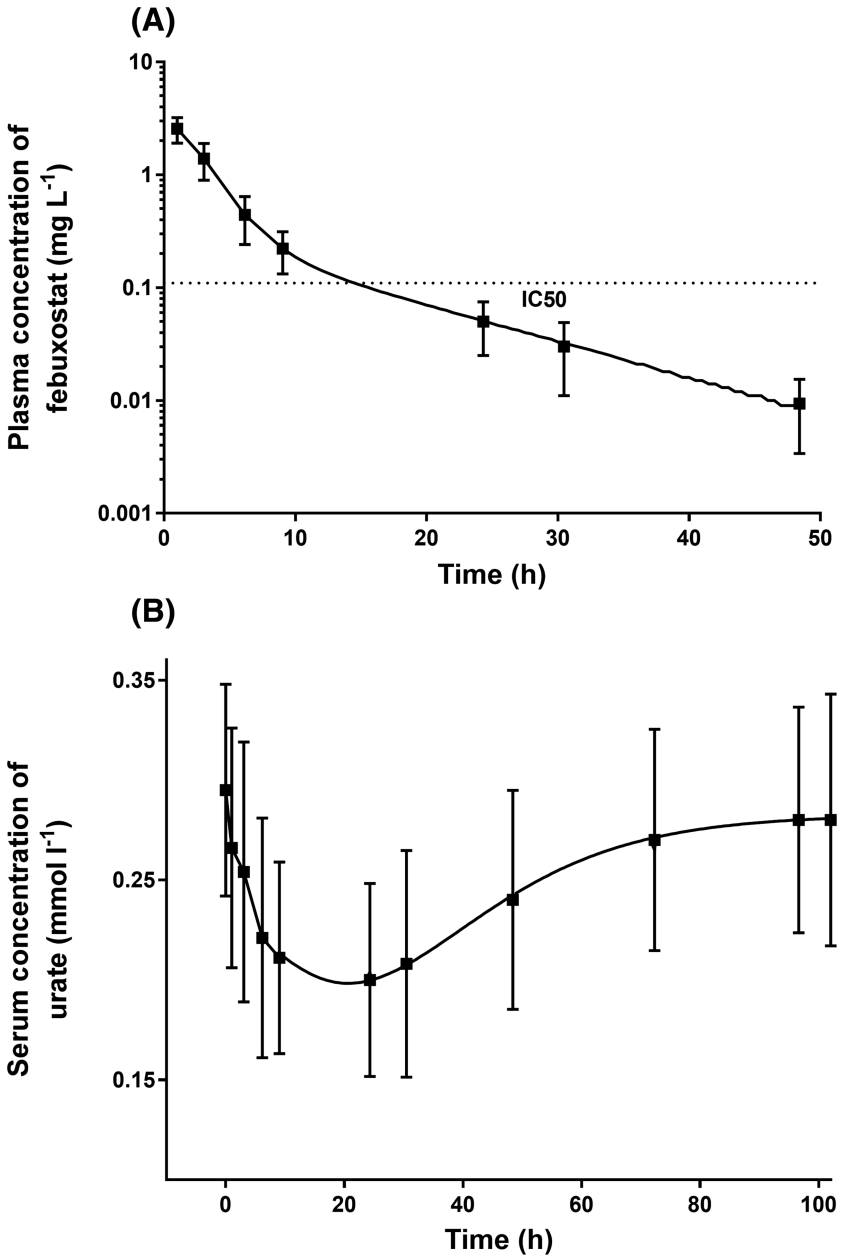

FIGURE 3 A, Time course of mean plasma concentrations of febuxostat and B, time course of mean serum urate concentrations after the administration of a single oral dose of febuxostat (80 mg) in healthy subjects (n = 9). The mean time course of plasma febuxostat concentrations was fitted to a two-compartment model (Equation 1). The simultaneous PK-PD model (Equation 2) was used to fit the mean concentrations of plasma febuxostat and serum urate. The lines are the predicted data and the squares and bars are the means and SD of observed data. The horizontal dashed line represents the IC_{50} of febuxostat 0.11 mg L^{-1}. Modelling was performed using MINIM version 3.0.8.[25]

to the nadir is due to the time taken to excrete the existing urate, which is consistent with the half-life of serum urate (approximately 24 hours[35]). Serum urate concentrations returned slowly to the baseline concentrations over about 3 days (72 hours). The slow recovery of urate can also be attributed to the long half-life of urate, three to five half-lives being the time to reach the pre-treatment concentrations assuming uniform production of urate by the body after the febuxostat-induced inhibition of XOR had reversed. Other possible but minor factors are the time for recovery of XOR activity *in vivo*, secondary to reversing the inhibition of the enzyme, and/or *de novo* synthesis of new XOR, and the continued hypouricaemic effect of the small concentrations of febuxostat in the terminal beta phase. Based on *in vitro* studies, Nishino et al. (2015)[36] reported that the value of the dissociation constant (K_d) of bovine milk XOR-febuxostat complex was too low to measure, even with a sensitive florescence detection method, but no confirmatory studies in humans have been published. The time course of inhibition and recovery of XOR activity *in vivo* with febuxostat requires further investigation.

The potent effect of febuxostat is confirmed by the very low IC_{50} value of 0.11 mg L^{-1} derived from the PK-PD model but this refers to total febuxostat in plasma. Febuxostat is 98-99% bound to plasma proteins.[37] Assuming that 1.5% of febuxostat is free in plasma, the unbound IC_{50} of febuxostat is about 1.61×10^{-3} mg L^{-1}. Assuming that therapeutic activity is achieved with 20% of the IC_{50}, febuxostat should have hypouricaemic effects down to approximately 0.32×10^{-3} mg L^{-1} of unbound febuxostat.

The IC_{50} value of febuxostat estimated in the present study lies between the values estimated from *in vitro* studies and an *in vivo* study conducted in gout patients. *In vitro* IC_{50} values of 1.4, 1.8 and 2.2 nmol L^{-1} were obtained from studies employing bovine milk,

TABLE 4 The estimated pharmacokinetic and pharmacodynamic (urate) parameters after a single dose of febuxostat in healthy subjects using the average concentrations of plasma febuxostat and serum urate concentrations (n = 9)

Pharmacokinetic parameters	Values
A	4.19 ± 0.69 mg $L^{-1,}$
B	0.15 ± 0.04 mg L^{-1}
α	0.40 ± 0.04 h^{-1}
β	0.06 ± 0.008 h^{-1}
Pharmacodynamic parameters	**Values**
Cu	0.289 ± 0.003 mg L^{-1}
k_u	0.053 ± 0.003 h^{-1}
IC_{50}	0.11 ± 0.09 mg L^{-1}

Data presented as mean ± SD.

Pharmacokinetic parameters and variations were determined by optimal fitting of the average plasma febuxostat concentrations to a two-compartment model (Equation 1, Figure 3, r^2 = 0.99). A PK-PD model (Equation 2) was fitted to the plasma febuxostat concentrations derived from Equation 1 and the average serum urate concentrations at times 0, 1, 3, 6, 9, 24, 30, 48, 72, 96 and 102 hours post-dose (Figure 3, r^2 = 0.99). Parameters and variations were determined by optimal fitting of theoretical equations using MINIM version 3.0.8.[25]

A and B, concentration terms; α and β, rate constants of elimination in the initial and terminal phases; *Cp*, plasma concentrations of febuxostat; Cu_t, serum concentrations of urate after dosage with febuxostat at time *t*; *Cu*, pre-dose concentration of serum urate; Cp_t, plasma concentration of febuxostat at time *t*; IC_{50}, plasma concentration of febuxostat that inhibits urate by 50%; k_u, rate constant of elimination of urate when its synthesis is blocked completely.

mouse liver and rat liver XOR enzymes, respectively.[38] Unfortunately, the IC_{50} of febuxostat on human XOR *in vitro* is unknown. Our *in vivo* IC_{50} estimate of unbound febuxostat of 1.61 × 10^{-3} mg L^{-} (5.0 nmol L^{-1}) is more than twice as high, assuming little protein binding in the in vitro preparations. An *in vivo* IC_{50} value of 0.24 mg L^{-1} was estimated from a population PK-PD compartmental model in patients with gout, the work so far in abstract form only and the model exhibiting a high coefficient of variation of 71%.[14] Again assuming 1.5% of plasma febuxostat is unbound, the unbound IC_{50} value of febuxostat in the gouty patients from the study of Khosravan et al. (2006) is approximately 3.6 × 10^{-3} mg L^{-1} (11.3 nmol L^{-1}). This value is 2.3-fold higher than the present estimated unbound IC_{50} *in vivo* and substantially greater than values from the *in vitro* studies noted. However, there are some differences between our method of PK-PD analysis and the method of Khosvaran et al. (2006).[14] The PK-PD model of Khosvaran et al. (2006) assumes that febuxostat-inhibitable XOR was in a peripheral compartment of urate. By contrast, our method assumes that the febuxostat-inhibitable enzyme is within the central volume of distribution of febuxostat. Despite these contrasts, it is evident that our compartmental method has not compromised the ability of the present model to produce a reasonable estimate of the IC_{50} of febuxostat. However, it is conceded that a mixed-effects, simultaneous population modelling approach would address a limitation of our two-staged approach to modelling the relationship between plasma febuxostat and serum urate, namely not accounting completely for between- and within-subject variability. Furthermore, employing unbound febuxostat concentrations for estimates of IC_{50} estimation would be preferable, especially in studies in patients where more variation in plasma albumin might be predicted.

In the present study, a single dose of febuxostat (80 mg) in healthy subjects achieved an approximately 35% maximal reduction in serum urate. This was comparable to that achieved with the same dose in healthy Chinese subjects (38%),[11] but was higher than that reported previously in American subjects (24%).[15] In the present study, females had lower baseline serum urate concentrations than male subjects, possibly due to the uricosuric properties of oestrogen in premenopausal women.[39,40] Females tended to have a greater percentage reduction in serum urate concentrations despite the absolute reductions in urate being similar between males and females, consistent with the lower baseline concentrations in women. Our data are in line with literature where the percentage reduction in urate is inversely proportional to the baseline urate concentrations.[41]

The estimates of renal urate excretion, namely urate excretion rate, ratio of urate to creatinine excretion rates, the Simkin Index and the 24 hour urinary urate output, changed in parallel with changes in serum urate concentrations induced by the single dose of febuxostat.

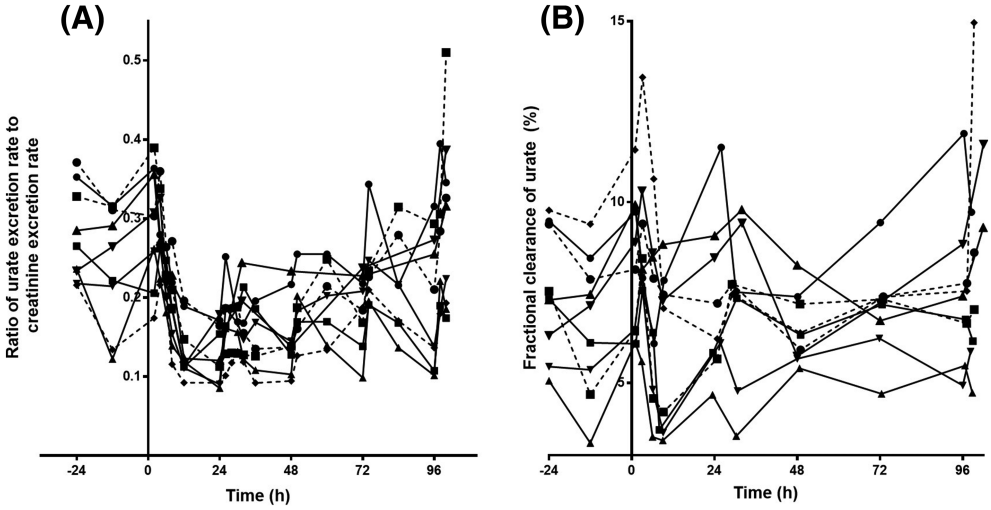

FIGURE 4 A, The ratio of urate excretion rate to creatinine excretion rate and B, The fractional renal clearance of urate (%) in healthy subjects (n = 9) at baseline and for up to 102 hours after the administration of a single dose of febuxostat (80 mg). Solid lines and dashed lines are male and female subjects, respectively. The other estimates of urinary urate excretion gave similar results to those in (A) and are presented in Supporting Information Figure S2a-c

TABLE 5 Mean FCU ± SD (coefficient of variation, %) before and after the single dose of febuxostat (80 mg)

Participant	Day 0[a]	Day 0-4[b]	Day 1[c]	Day 2-4[d]
ID32	7.4 ± 0.1 (0.9)	8.5 ± 1.0 (11.7)	8.9 ± 0.6 (6.8)	8.3 ± 1.1 (13.8)
ID33	6.7 ± 0.4 (6.0)	8.5 ± 1.6 (19.0)	8.7 ± 1.0 (11.2)	8.6 ± 2.0 (23.2)
ID34	9.0 ± 0.5 (5.6)	8.9 ± 1.7 (19.1)	8.6 ± 1.9 (21.5)	9.2 ± 1.7 (18.0)
ID35	6.7 ± 0.6 (9.0)	6.4 ± 1.0 (15.6)	5.9 ± 1.3 (21.2)	6.8 ± 0.5 (6.8)
ID36	4.2 ± 0.9 (21.4)	4.7 ± 0.9 (18.9)	4.7 ± 1.1 (23.3)	4.8 ± 0.7 (14.7)
ID37	5.4 ± 0.04 (0.8)	5.6 ± 1.1 (18.9)	5.8 ± 1.5 (25.2)	5.5 ± 0.6 (10.1)
ID38	6.1 ± 1.4 (23.0)	6.5 ± 1.2 (18.9)	5.9 ± 1.5 (25.8)	7.2 ± 0.4 (4.9)
ID39	8.6 ± 0.8 (9.3)	7.8 ± 0.9 (11.0)	8.02 ± 0.8 (9.9)	7.5 ± 0.9 (11.7)
ID40	9.6 ± 0.2 (10.4)	9.3 ± 2.8 (30.6)	9.8 ± 2.7 (27.9)	8.7 ± 3.1 (35.9)
Male subjects (n = 6)	6.6 ± 1.4 (21.4)	7.1 ± 2.04 (28.8)	7.1 ± 2.1 (29.8)	7.2 ± 2.04 (28.5)
Female subjects (n = 3)	8.1 ± 1.5 (18.2)	7.9 ± 2.2 (27.7)	7.9 ± 2.4 (31.0)	7.8 ± 2.01 (25.7)
All subjects (n = 9)	7.1 ± 1.7 (23.5)	7.3 ± 2.1 (28.9)	7.4 ± 2.3 (30.7)	7.4 ± 2.1 (27.8)

A total of 11 blood specimens were collected from each subject, immediately before dosing and at the following times after administration of a single dose of febuxostat: 1, 3, 6, 9, 24 (±2), 31 (±1), 48 (±2), 72 (±2), 96 (±2) and 102 hours. Urine (total catch) was collected before the administration of febuxostat 80 mg (−24 to −12 hours and −12 to 0 hours) and then for the duration of the study up to 102 hours after the administration of febuxostat 80 mg, the latter in timed, divided collections. For the two healthy male subjects who underwent the study procedures twice, urine collections for each individual were performed at −24 to −12 hours and −12 to 0 hours and then 0-2, 2-4, 4-6, 6-8, 8-12, 12-24, 25, 30, 48, 72, 96 and 101.5-102.5 hours after drug administration to calculate FCU. The 25, 30, 48, 72 and 96 urine collections were spot tests.

[a]Pre-dose FCU in individual subjects (average of two estimates).

[b]FCU estimates throughout the whole study duration including the pre-dose and 10 samples obtained after the administration of a single dose of febuxostat for each participating subject, a total of 99 FCU estimates for all nine participating subjects.

[c]FCU estimates during the first 24 hours post-dose, five samples obtained for each subject after the administration of a single dose of febuxostat.

[d]FCU estimates for days 2-4 of the study, five samples obtained after the administration of a single dose of febuxostat for each subject during this period.

TABLE 6 FCUs (mean ± SD) and the differences in FCUs from the baseline FCU (medians and 99.5% confidence intervals for absolute differences) at multiple times after the single dose of febuxostat 80 mg given to healthy subjects (n = 9). Signed rank Wilcoxon tests comparing the post-intervention differences in FCU with the baseline FCU, respectively

Time post-dose to mid-point FCU urine collection	Mean ± SD FCU	Median difference in FCU from baseline (99.5% confidence interval)	Adjusted *P* values for comparisons of median difference in FCUs from baseline (Bonferoni correction for 10 pairs)
0 h (pre-dose FCU)	7.1 ± 1.7	0	NA
1 h	8.1 ± 1.9	1.1 (−2.7, 0.6)	0.4
3 h	8.7 ± 2.1	−1.6 (−3.9, 1.1)	0.3
6 h	6.8 ± 2.2	0.5 (−1.9, 2.9)	1.0
9 h	5.9 ± 2.0	1.2 (−1.6, 3.0)	0.5
24 h	7.2 ± 2.0	−0.2 (−2.5, 3.4)	1.0
30 h	7.3 ± 1.9	−0.2 (−2.7, 2.1)	1.0
48 h	6.5 ± 0.9	0.7 (−1.2, 3.2)	1.0
72 h	7.1 ± 1.2	−0.1 (−1.2, 2.2)	1.0
96 h	7.5 ± 1.9	−0.3 (−2.9, 2.0)	1.0
102 h	8.7 ± 3.0	−1.01 (−5.4, 0.6)	0.4

This was expected as the amount of urate presenting to the kidney was reduced after the inhibition of urate production by febuxostat (80 mg). The excretion of urate subsequently increased gradually as the amount of urate presenting to the kidney recovered to the pre-dose concentrations.

We did not detect a systematic effect of a single dose of febuxostat (80 mg) on FCU over the time course of the study, nor on urate or creatinine clearance. The lack of apparent effect on FCU, a variable that estimates the efficiency of renal clearance of urate, is not unexpected with a drug that reduces urate synthesis through the inhibition of XOR. However, Liu et al. (2017)[16] reported that febuxostat 40-80 mg/day reduced FCU in healthy and gouty subjects after chronic administration for 7 days.[16] Although the numbers of subjects in our present prospective study are small, multiple collections of blood and urine allowed a more detailed examination of the effect of febuxostat on FCU. The coefficient of variation for FCU was less than 31% at any time point in the present study, which is consistent with the literature values obtained in healthy subjects (28%).[18]

Furthermore, the intra-subject coefficient of variation for the current FCU data of less than 20% (with the exception of one subject) is similar to that reported by Kannangara et al. (2012) (16%).[18] However, further studies on the effects of febuxostat on FCU in individuals taking this drug chronically are needed.

Febuxostat was recently identified by Miyata et al. (2016)[42] to be an inhibitor of the ATP-binding cassette transporter, subfamily G2 (ABCG2), a urate transporter in the gut and the kidney that contributes to the intestinal and urinary secretion of urate. However, it is unlikely that febuxostat affects FCU through the inhibition of ABCG2 greatly since there is little expression of ABCG2 in the human kidney in comparison with the small intestine and the liver.[43] Furthermore, it was demonstrated that loss of function in ABCG2, specifically the Q141K polymorphism, was not associated with an effect on FCU in a study that was conducted in people with hyperuricaemia (n = 448) or normouricaemia (n = 344).[44] Kannangara et al. (2015)[44] concluded that loss of function in ABCG2 contributed to hyperuricaemia by reducing the extrarenal (gut) clearance of urate rather than an effect on FCU. Additionally, it has been noted that febuxostat had no effect on the transport of urate by urate anion transporter 1 (URAT1)[42] responsible for the bulk of reabsorption of urate in the renal proximal tubules.[45]

5 | CONCLUSION

The current study used a two-compartment model to elucidate the pharmacokinetics of febuxostat in healthy subjects. Febuxostat displayed a negative hysteresis, with the change in serum urate concentrations lagging behind the time course of plasma febuxostat. While a single dose of febuxostat reduced the rate of excretion of urate, specifically it did not reduce the FCU in healthy subjects. A PK-PD model fitted mean plasma febuxostat and serum urate concentrations well. This model may have clinical utility, but more detailed evaluation is required in patients with gout who are being treated with febuxostat chronically.

ACKNOWLEDGEMENTS

The authors thank Dr Emma Tay and Dr Jonathan Brett for their assistance in performing the medical and physical examination on the participants prior to entry into the study and at the study exit. Finally, the authors thank the healthy subjects who participated in this study. The present work was funded by The Lexy Davies Bequest at St Vincent's Hospital, Sydney. The funding body had no input in the design of the study, collection, analysis, and interpretation of the data and writing of the manuscript.

COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTIONS

All authors contributed to the inception, design of the study and writing of the present manuscript. Additionally, B.K., G.G., Z.L., K.P. and R.D. were involved in the data analyses. Each author contributed to important intellectual content during drafting and/or revision of the manuscript and accepts accountability for the overall work.

DATA AVAILABILITY STATEMENT

We do not have Ethics Committee or research subject participants permission to share study data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Kamel B, Graham GG, Stocker SL, et al. A pharmacokinetic-pharmacodynamic study of a single dose of febuxostat in healthy subjects. *Br J Clin Pharmacol*. 2020;86:2486–2496. https://doi.org/10.1111/bcp.14357